Tumor Res., **13**, 1-13, 1978

Tumor Dormancy: A Review

Elias A. K. ALSABTI

Royal Scientific Society Amman, JORDAN

An important objective of cancer therapy today is to eliminate the few residual tumor cells remaining in the body following treatment with surgery, radiation, chemotherapy or immunotherapy. In many cases, failure of such therapy is evidenced by the reappearance of overt neoplasia months or years after clinical remission. Hadfield (1954) pointed out that when the interval between the excision of some primary malignant tumor and the appearance of secondaries exceeds 2 and reaches 4, 14 or 40 years like carcinoma of breast, melanoma or colon cancer, it is difficult to believe that cellular proliferation in the residual tumor has been continuous, and it seems almost impossible to escape the conclusion that these cells have been in a state of temporary mitotic arrest. Hadfield employed the term dormant to describe the malignant cells which, although remaining viable for relatively long periods, show no evidence of multiplication during this time, yet retain all their former and vigorous capacity to multiply.

Evidence for the existence of tumor cells in a dormant state in clinically normal persons comes from histological studies such as that of Beckwith and Perrin (1963) who found in unselected autopies of children up to the age of three months, small foci of cells resembling typical neuroblastomas in the adrenals at a frequency that was 40 to 50 times that expected from the incidence of clinical adrenal neuroblastoma. Mortensen (1955) found that 49.5% of 821 thyroids known to be clinically normal during life contain nodules, 17 of which were histologically malignant. Since the incidence of clinical thyroid cancer is about 6 per million, this finding of 2% incidence of histologically malignant lesions in unselected autopsies is much greater than would have been predicted. Similar studies have been performed on carcinoma of prostate by Ashley (1965), Munsie and Foster (1968), and Montgomery (1961).

Following the introduction of smear cytology as a technique for early diagnosis of cervical carcinoma, vast numbers of "carcinoma in situ" cases were reported. Again it is statistically evident that a large proportion of the precarcinoma lesions never develop into clinical disease. Clearly, some process, immunologic or otherwise, must act to restrain these precancerous cells

Reprint Rexuests: Dr. Elias Alsabti c/o Mrs. W. Aljaff Flat No. 5. Norfolk Terrace Brighton BN 1 3 AD ENGLAND.

from active growth to overt neoplasia (Green, 1966; Coppleson and Reid, 1968; and Ashley, 1966).

Additional clinical evidence to substantiate the presence of recurrence of solid tumors and leukemias in patients years after apparently successful treatment of the primary neoplasia. Several such examples have been noted by Taylor (1959) of apparently successful removal of cancerous breast tissue from humans and the reappearance of such cancer 20–40 years later. These reports suggest that residual tumor cells do survive the protracted periods of clinical remission, but do not rule out *de novo* recurrence of the same type of cancer due to a long lasting predisposition of the patients' cells to malignant transformation.

An understanding of tumor dormancy requires identification of the host factors that act to transiently restrain residual tumor cells from outgrowth and the mechanisms involved in the final breakthrough to overt neoplasia. However, a few animal models have been developed for study of tumor dormancy.

Tumor dormancy is a state of co-existence in which tumor cells persist in a clinically normal host for prolonged periods of times. This state of dormancy is characterized by two remarkable features:

1) Tumor cells are not destroyed by the host's natural defence mechanisms.
2) Tumor cells do not grow out rapidly to form a clinically overt tumor.

Although tumor dormancy has been recognized as a clinical problem for many years, very few examples of tumor dormant states in experimental animals have been reported. This may be due to the fact that experiments demonstrating resistance of immune animals to tumor cell challenge are usually of short duration and frequently terminated long before putatively dormant cells can emerge to produce overt tumors. In such experiments, resistance of mice to tumor cell outgrowth may often be associated with prolonged tumor dormancy rather than tumor cell rejection. However, Schabel (1977) suggested that tumor dormancy is not commonly seen in the laboratory since most tumor systems are usually selected for their rapid and consistent growth with uniformly high mortality.

The first animal model for tumor dormancy was reported in 1959 (a) by Fisher and Fisher. These investigators demonstrated that non-inbred rats inoculated intraportally with as few as 50 Walker 256 carcino-sarcoma cells did not develop the expected signs of hepatic tumors when examined five months post-inoculation. However, if three months after tumor inoculation these rats were subjected to repeated laparotomy and liver examination, 100% of the rats developed hepatic tumors within a couple of weeks of

the mainpulation. These results indicated that tumor cells may remain dormant in the liver in a viable state until growth is triggered by a stimulus associated with hepatic trauma.

Gordon-Taylor (1959) referred to the tumor dormancy state as the incomputable factor in cancer prognosis. He and Boyd (1960) have cited many clinical cases of recurrent tumors developing years after apparently successful eradication of primary tumors originating in a variety of organs. In many of these cases, especially mammary tumors, recurrences have appeared in the scar of the previous surgical incisions. Recurrences were also observed shortly after attacks of acute bacterial infections, non-oncological surgery involving other organs, during pregnancy, and also in apparently healthy individuals.

In 1971, Sugarbaker redemonstrated tumor cell dormancy with the Walker-256 tumor cell in non-inbred (allogeneic) rats as previously described by Fisher and Fisher (1959 a). However, attempts to extend the study of tumor dormancy to a syngeneic system utilizing rats and strongly immunogenic carcinogen-induced sarcoma were unsuccessful. Sugarbaker has interpreted these results to suggest that weakly or non-immunogenic tumors may have a greater ability to survive in peaceful coexistence with the host than highly immunogenic tumors. Thus, immunogenicity of the tumor cell may be an important factor in establishing tumor dormancy.

Gimbrone, *et al.* (1972) in a study of tumor dormancy, produced avascular carcinomas by anterior eye chamber implantation in a susceptible rabbit strain. Unable to elicit new capillary ingrowth, these small spherical tumors appeared to survive by simple diffusion, and remained dormant for as long as six weeks. Gimbrone, therefore, introduced a new concept of dormancy in which growth arrest of a population of neoplastic cells is due to lack of vascularization.

Noble and Hoover (1974) reported that hormone-dependent adrenal, mammary and other tumor cells, transplanted to unconditioned male or female animals, remained viable but dormant until they were stimulated by estrogen, even after periods of 14 months of dormancy. Animals bearing these dormant cells were indistinguishable from normal animals and presumably would surivive a normal life span without tumor growth. Thus, Noble and Hoover have observed that estrogen-dependent tumors remain dormant in the absence of estrogen or grow proportionally to the amount of estrogen given to the host.

Eccles and Alexander (1975) found that rats in which primary tumor transplants had been surgically removed were clinically normal for many months. However, these clinically normal rats, when subjected to whole

body irradiation or thoracic duct drainage, developed overt tumors from metastases which had persisted in a dormant state following surgical removel of the primary implant.

Yu, *et al.* (1977) reported that I. V. inoculation of small numbers of highly metastatic carcinoma results in formation of a carcinoma in the lung. Through the use of localized radiotherapy and a radio-protective drug, these investigators apparently reduced the tumor burden in the lung, and assumed, by the prolonged survival of mice, that they had eliminated the few remaining tumor cells. However, long term observation indicated that between 120-160 days after treatment, more than 90% of the mice died of metastasis to the brain, kidney, liver, breast and other organs. These results were interpreted by Yu, *et al.*, to represent a true example of tumor dormancy.

To date only a few animal models have been developed for the study of tumor dormant states, however tumor dormancy in experimental animals may be a frequent occurence which goes unrecoginzed. Wheelock (1978) suggested that tumor dormancy is not commonly seen in the laboratory, since many tumors are usually selected for rapid and consistent growth with uniformly high mortality. Also experiments to test resistance of mice to tumor cells are often terminated as soon as resistance is demonstrated and animals are not permitted to survive long enough for dormant tumor cells to emerge to produce overt tumors. Thus, resistance to tumor cell challenge may often be associated with tumor dormancy rather than total tumor cell rejection.

TUMOR SUPPRESSION AND DORMANCY

The ability to suppress tumor formation had been shown to involve the action of a number of host immune and non-immune effector mechanisms (Cerottini and Brunner, 1974). Macrophages have been shown to be cytotoxic for tumor cells (Evans and Alexander, 1972; Zembula, *et al.*, 1973; and Keller, 1974 b) and this cytotoxicity is believed to be tumor specific but non-immunologic and related to abnormal growth properties rather than the antigenic composition of target cells (Hibbs, *et al.*, 1972). In contrast, immune effectors are directed against TAAs on the cell surface. However, in the tumor dormant state, tumor cell outgrowth is suppressed but the tumor cell itself is not eradicated from the host. Maintenance of tumor dormancy may involve the loss of exposed cell surface targets for host effector mechanisms. The most likely explanation for such loss is antigenic modulation, i. e., the reversible disappearance of membrane antigens upon antibody contact (Old, *et al.*, 1968). One may speculate that during tumor dormancy expression of an oncogene is suppressed by action of host antitumor mechanisms

acting at the cell surface. Alternatively, some surface receptors responsible for regulation of cell division that are usually concealed during tumor progression may become unmasked during antigenic modulation, thereby leading to recovery of control of cell division by the host. Finally, tumor dormancy could be established by the action of cytostatic rather than cytolytic host mechanisms.

Goldstein, *et al.* (1973) reported the production of a cytophilic but non-cytotoxic antibody in L5178Y immunized DBA/2 mice. The possible cytostatic effects of this antibody are not yet known. Antibody directed against surface TSTA may have tumor enhancing properties (Kaliss, 1958) possibly as a result of binding to antigen and blockade of the cytotoxic lymphocyte response (Sjogren, *et al.* (1971). Such tumor enhancing antibodies are clearly not operative during the tumor dormant state but may be involved when tumors break dormancy and grow to overt neoplasia. The existence of "unblocking" serum factors has been proposed by Hellstrom and Hellstrom (1970) as a means of explaining the regression of murine sarcomas in mice that have produced blocking antibody during the tumor progressor phase. Implicit in maintenance of the dormant tumor system, however, is suppression of tumor cells without total cytolysis and attention must therefore be directed to the host factors that can produce a long lasting cytostatic effect.

In addition to those of Old, *et al.* (1968) a few studies have been performed on the non-cytolytic effects of antibody reacting with specific antigen at the cell surface. Joseph and Oldstone (1974) describe capping and shedding of measles virus antigen from the surface of infected cells following their exposure to virus specific antibody. Antibody directed against polyoma virus determined tumor associated cell surface antigens can induce regression of polyoma tumors (Bansal and Sjogren, 1973); however, persistence of the virus genome in a dormant state following tumor regression was not studied. The realization that cellular elements of the immune system may play an important role in cytostasis has stemmed mainly from the work of Keller (1973, 1974 b, 1975, 1975 a, b) who showed that non-specifically activated macrophages can exert a cytostatic, non-cytolytic effect upon tumor cells. Recently, spleen cells (Senik, *et al.* 1977) and lymph node cells (Chia and Festenstein, 1973) from tumor-bearing mice have been shown to possess non-cytolytic, tumor suppressive activity. In all studies, the putative cell responsible for cytostasis is a non-T-cell which is adherent, most probably a macrophage, and which can exert growth restrictions upon many different unrelated tumor cells. To our knowledge, however, no cytostatic effects have as yet been described by T-cell dependent or antibody-mediated B-cell effector mechanisms. However, antibody-dependent lymphocyte tumor cell interac-

tion could conceivably lead to cytostasis as well as the demonstrated cytotoxic effects (Lamon, 1974). Of interest here is the demonstration that IgG but not IgM has been shown to be highly effective in stimulating lysis by non-immune effector cells (MacLennan, 1972). The cytostatic effects of IgM-dependent lymphocyte tumor cell interaction has not been determined.

TUMOR EMERGENCE

Nature abounds with examples of the escape of biological systems from restrictions imposed by their environment. In tumor suppressor systems, many routes of escape have been identified (Klein, 1961, 1972 and Bonmassar, *et al.*, 1974), and more undoubetedly will be discovered.

Tumor escape may result from impairments in immune mechanisms due to senescence, a phenomenon demonstrated both with regard to tumor rejection and humoral antibody formation (Celada, 1968 and Stjernsward, 1968). The high incidence of tumor emergence during prolonged immuno-suppression in man is note worthy (Penn and Starzel, 1972). The low incidence of tumors in "nude" mice argues against this escape route, although B-cells and macorphages in these mice may exert strong tumor suppressive effects. Blockade of an effective host anti-tumor mechanism *in vitro* has also been proposed as a posible mechanism for tumor escape *in vivo*. Evidence is increasing that the blocking factor is not an antibody as originally thought, but rather an antigen or antigen-antibody complexes (Sjogren, *et al.*, 1971). Thus, blockade of immune systems and subsequent tumor escape may be due to a shift of balance from antibody excess to antigen excess. This blockade theory can be readily adapted to tumor emergence from a prolonged dormant state, since tumor outgrowth probably occurs in the presence of an immune response. In contrast, such blockade in non-immune hosts probably plays no role. The "sneaking through" hypothesis first proposed by Old, *et al.* (1962) describes a proliferation of small numbers of tumor cells in the presence of a sluggish immune response. It is not readily applicable to tumors emerging from a dormant state in which immune mechanisms have already been mobilized.

Tumor escape could also result from the absence of cell surface tumor-associated antigens. This phenomenon could account for rapid growth of primary tumors and could explain outgrowth from the tumor dormant state following antigenic modulation of TAA or immunoselection. The lack of surface antigen could also be due to concealment of TAA within glycoprotein that have been demonstrated to coat the surface of certain tumor cells (Simmons, *et al.* 1971).

Emergence of tumors in mice following a long tumor dormant period

may be due to induction of a new tumor of host origin by endogeneous viruses released from the original tumor rather than outgrowth of the original tumor dormancy. Igel, *et al.* (1969) and Ball and McCarter (1971) reported production of murine endogenous viruses following treatment with chemical carcinogens. C-type particles have been observed in L5178Y cells by electron microscopy (Wheelock, 1968). Filterable agents can induce a variety of tumors (Toth, 1963) and inoculation of X-irradiated thymic dimethylbezanthracene-induced lymphoma cells into mice induced thymomas of host origin (Chan and St. C. Cinclair, 1972).

Concomitant and sinecomitant immune control of tumor metastases

Immunologic, nutritional and hormonal, Noble and Hoover (1974) factors have all been implicated in the establishment of tumor dormancy in animals. In man, however, the prolonged suppression of tumor metastasis and the emergence of a dormant state is probably mediated by a sinecomitant immune response to tumor-associated antigens. Erhlich in 1906 and Bashford in 1908 described a phenomenon in which mice bearing a progressively growing tumor implant rejected a second implant of the same tumor. Bashford termed this phenomenon, "conconmitant immunity". Investigation into this type of antitumor resistance was not renewed until 1964 when Riggins and Pilch demonstrated immune resistance to an MCA-induced fibrosarcoma when murine hosts received a tumor challenge 7 days after removal of the primary tumor mass. Results from these and other experiments indicated that the outgrowth of primary tumors can immunize the tumor-bearing hosts and protect them against subsequent challenges (reviewed by Vaage, 1971).

An important aspect of concomitant immune states is the size of the primary tumor mass at the time of secondary implantation. Barski, *et al.* (1969) utilizing three different syngeneic transplantable tumor cell lines, demonstrated the appearance of specific immunologic activity after primary implantation of the tumor. Progressive tumor growth, however, consistently resulted in either a decreased or complete disappearance of host immune reactivity against the tumor. This period of tumor-directed immunologic hyporesponsiveness or even paralysis has been termed the "eclipse period". Subsequent tumor cell challenges given during this critical "eclipse" time interval are not rejected. This "eclipse" may result from a blockade of the effector immune cells by either tumor-associated antigens, antibodies or antigen-antibody complexes (Sjogren, 1971; and Currie, 1972) or by immune suppressor cells (Broder, 1978).

The temporal immunologic anergy in concomitant immune systems was overcome by excision of the primary tumor mass followed by reimplantation several days later. Fisher, *et al.* (1970) termed this variation "sinecomitant

immunity". The most critical factor in sinecomitant immune systems is the length of time between excision and challenge. A less than optimum immune response against the challenge tumor cells is produced if they are administered either too soon or two long after excision of the primary tumor.

The sinecomitant murine tumor dormancy model

Weinhold (1977) had utilized the sinecomitant immunization procedure to develop a murine model in which 100% of immunized mice resist the rapid outgrowth of an intraperitoneal L5178Y cell challenge administered 7 days after subcutaneous tumor nodule excision. This resistance appears to be associated with a state of tumor dormancy since mice challenged with L5178Y cells remain clinically normal for 90–360 days, while during this period, viable tumor cells can be isolated from their peritoneal cavity and grown *in vitro*.

Establishment of the tumor dormancy state in sinecomitant murine model (Wheelock, 1978) follows a tumor cell growth and lysis phase, in which the challenge tumor cells first divide rapidly, with the majority of cells then lysed by host peritoneal cells. This growth and lysis phase is followed by prolonged tumor dormancy in which the number of tumor cells in the peritoneal cavity is maintained at about 10,000 until termination of tumor dormancy, signaled by rapid outgrowth of the tumor cells.

The murine sinecomitant tumor dormancy model may bear resemblance to tumor dormancy in man, where metastatic tumor cells may remain dormant at distant sites for long periods of time after excision of the primary tumor, and then suddenly grow out to become overt tumor. A probable dormant state explanation for both the human and the murine tumor is that an immune response, elicited by the primary tumor prior to its excision, has suppressed distantly located microscopic nests of tumor cells to a dormant state. The eventual outgrowth of overt tumors from these cells may be associated with the onset of derrangement in immune competence in the host. The mechanism of such derrangement is yet to be ascertained.

Diagnosis of tumor dormant state

A tumor dormant state can be diagnosed prospectively with a great deal of effort by identification of tumor cells in tissue sections or their isolation from a host who will remain clinically normal for a predetermined period of time. The identification of tumor product markers (such as CEA) sometimes several months prior to clincal detection of colorectal cancer can also suggest a tumor dormancy state. Retrospectively a tumor dormant state can be determined by identification of emerging tumor cells as progeny of the original tumor cells. This strict latter criterion is necessary since recurrences of tumors or years after elimination of the primary tumor could be

due to retransformation of normal cells by carcinogenic to etiological agents, as strongly suggested in patients with acute leukemia following bone marrow transplantation (Elfenbein, 1978).

Identification and recovery of tumor cells

The identification of tumor cells that are embedded in a matrix of normal cells can be exceedingly difficult, and their separation from normal cells may be impossible. This is due to the fact that the dormant tumor cells are few in number and may be transiently differentiated so as to resemble normal cells norphologically. The isolation of dormant tumor cells by *in vitro* culture appears more feasible. The tumor cells which may grow out in culture do not reflect the true cytokinetic state of the cells which exist in the host. A complicating factor in the recovery of dormant tumor cells from a host is that such cells are not necessarily embedded in the normal tissue matrix adjacent to the original primary tumor site, but may be present at a distant site to which they have metastasized. Futhermore, although it is possible, in experiemented animal models, to culture all organs and body compartments, this is impractical with patients. Isolation attempts from patients in clinical remission after apparent successful treatment of a primary tumor is therefore limited to "second look" exploratory procedures where in the absence of gross tumor regional lymph nodes, bone marrow or tissues from the vicinity of the original primary tumor are blindly samples. The outgrowth of tumor cells *in vitro* may be accomplished since the normal contaminating cells may not have the same rapid proliferative characteristics. However, the presence of sufficient tumor-suppressing host cells in the culture may inhibit the outgrowth of tumor cells. The latter can be expected to be present since the tumor cells have been under growth restraint *in vivo*. Specific measures may have to be taken to suppress antitumor host cell activity during culture *in vitro* (Wheelock, 1978). Such tumor cell isolations have been made by peritoneal lavage in mice surviving for more than three months thereafter without overt ascitic tumors. They attribute this outgrowth *in vitro*, but not *in vivo*, to two possible factors.

a) *Cell Density*

The cultivation of 10^6 peritoneal cells from a tumor dormant mouse usually fails to yield tumor cell outgrowth, whereas wells containing 10^5 cells have consistently yielded proliferating tumor cells. This observation can be explained by a difference in the overall cell density in culture. A high cell density forces continued close contact between host effector and tumor cells resulting in suppression of tumor growth. In contrast, a low cell density permits tumor cells to escape such close contact, resulting in tumor cell

outgrowth. Unknown as yet, but extremely important is the density and ratios of host effector and tumor cells in the tumor dormant host.

b) *Cytostasis and escape thereform*

Host cells which exhibit tumor suppressive activity *in vivo* have been shown to lose such activity rapidly when placed in culture *in vitro*. Remington (1976) has shown that macrophages from immune mice can exert a cytostatic effect on tumor cells for only 24–48 hours after being removed from the mouse. Such escape of tumor cells from the cytostatic effects of host cells may be the basis for tumor cell outgrowth *in vitro* in our sine-comitant tumor dormant model.

Identification of emerging tumor cells

The identification of tumor cells that emerge at the end of a tumor dormant period as progeny of the original cells rather than as newly induced in the host by oncognic viruses or chemical carcinogen, necessitates the presence of markers on such cells. Wheelock had been able to perform such an experiment by comparing sex chromosomes in the original and emerging cells. Since his original L5178 cells were induced in a female DBA/2 mouse, they bear XX chromosomes. He had induced a tumor state with such cells in male DBA/2 mice. A karyotype analysis of tumor cells growing out at the end of the tumor dormant period reveals them to be female, proving that they were progeny of the original L5178Y cells. He had also established a tumor dormant state in DBA/2 $\times$ C517B1/6 F_1 hybrid mice with DBA/2 derived L5178Y cells. Tumors emerging after a prolonged period of dormancy contain DBA/2 but not C517B1/6 histo compatible antigens, also proving them to be progeny of the original 5178Y cells.

Similar immunogenetic approaches are clearly not possible in clinical situations. However, other markers such as tumor-derived antigens, isoenzymes and perhaps ectopically produced hormones may all be useful in a comparison between the original and the emerging cells.

Concluding Remarks

An important factor influencing tumor dormancy is the anatomical location of the tumor cell. Gimbrone (1972) have demonstrated that tumor cells deprived of direct vascularization do not divide but remain viable and retain their neoplastic potential. A clinical phenomenon possibly related to Gimbrone's model is the malignant cell that remains dormant for many years in the scar tissue following a surgical procedure and then grows out to an overt tumor.

Tumor cell-host interactions resulting in a tumor dormant state can best be analyzed *in vitro*. The tumor cell itself may change during the

kinetics of establishment, maintenance and termination of the tumor dormant state. Therefore, a study of the tumor dormant state requires analyses and comparisons of three types of tumor cells; the original, the dormant and the emerging. The parameters in these cells that should be evaluated are the expression of endogenous antigenic and non-antigenic markers, the mitotic state and rate, the invasive and metastatic properties and the susceptibility and responses to various immune effector components.

The identification of host cellular and soluble components required for establishment of the steady state cell kinetics that characterize tumor dormancy can best be accomplished by the development of an *in vitro* model. However, this is a most difficult problem since the culture conditions themselves can alter the tumor suppressive activity of host factors. It is possible, furthermore, that a sequence of host reponses to proliferating tumor cells is required to suppress them to a dormant state. An alternate approach is to activate tumor growth in tumor dormant mice by ablation of specific immune or non-specific host components, or by direct stimulation of tumor cells using agents that can open G_0, G_1 or G_2 barrier (Gelfant, 1977).

An understanding of the tumor dormant state is central to the control of cancer. The ability to suppress the malignant expression of a cell, to convert it to a mitotically arrested dormant cell, and to maintain it in that dormant state constitutes effective treatment of cancer. Furthermore, the ability to control the emergence from the dormant state of those few residual tumor cells which have escaped primary cancer therapy by cell cycle specific adjuvant therapy directed against S Phase may constitute a cure of cancer. Above all, an understanding of the biochemical, physiological and immunological differences among normal, malignant and tumor dormant cells should provide insight into the entire basis of oncogenesis.

ACKNOWLEDGEMENTS

Many thanks for His Royal Highness Crown Prince Hassan for his financial support and encouragement.

REFERENCES

1. ALEXANDER P. A.: In "Fundamental Aspects of Metastasis". North-Holland Publishing Co., pp. 227–239 (1976).
2. AL-GHAZZOULI I. K., DONAHOE R. M., HUANG K. Y., SASS B., PETERS R. L. and KELLOFF G. J.: J. Immunol. **117**, 2239–2248 (1976).
3. ASHLEY D. J.: J. Path. Bact. **90**, 217–224 (1965).
4. ASHLEY D. J.: J. Obstet. Gynaec. Brit. Comm. **73**, 372–381 (1966).

5. BALL J. K. and MCCARTER J. A.: J. Natl. Cancer Inst. **46**, 751–762 (1971).
6. BANSAL S. C. and SJOGREN H. O.: Fed. Proc. **32**, 165–172 (1973).
7. BARSKI G. and YOUN J. K.: J. Natl. Cancer Inst. **43**, 111–121 (1969).
8. BASHFORD E. F.: Third Scientific Report of the Imperial Cancer Research Fund, Taylor and Francis Ltd, London, pp. 262–292 (1908).
9. BECKWITH J. B. and PERRIN E. V.: Am. J. Pathol. **43**, 1089–1104 (1963).
10. BONMASSAR E., MENCONI E., GOLDIN A. and CUDKOWICZ G.: J. Natl. Cancer Inst. **53**, 475–479 (1974).
11. BRODER S., MUUL L. and WALDMANN T.: Cancer **61**, 5–11 (1978).
12. CELADA F., SCHMIDT D. and STROM R.: Immunology **17**, 189–198 (1969).
13. CEROTTINI J. C. and BRUNNER K. T.: Adv. Immunol. **18**, 67–132 (1974).
14. CHAN P. L. and St. C. SINCLAIR: J. Natl. Cancer Inst. **48**, 1629–1640 (1972).
15. CHIA E. and FESTENSTEIN H.: Europ. J. Immunol. **3**, 483–487 (1973).
16. COPPLESON M. and REID B.: Lancet **2**, 873 (1968).
17. CURRIE G. A. and BASHAM C.: Brit. J. Cancer **26**, 427–437 (1972).
18. ECCLES S. A. and ALEXANDER P.: Nature **250**, 667–669 (1974).
19. EHRLICH P.: Arbeiten aus dem Kungl Institut für experimentelle Therapie zu Frankfurt am Main. **1**, 65–80 (1906).
20. ELFENBEIN G. J., BROGAONKAR D. S., BIAS W. B., BURNS W. H., SARAL R., SENSENBRENNER L. L., TUTSCHKA P. J., ZACZEK B. S., ZANDER A. R., EPSTEIN R. B., ROWLEY J. D. and SANTOS G. W.: Blood **52**, 627–637 (1978).
21. EVANS R. and ALEXANDER P.: Nature **236**, 168–170 (1972).
22. FISHER B. and FISHER E. R.: Ann. Surg. **150**, 731–739 (1959).
23. FISHER B., SAFFER E. A. and FISHER E. R.: Proc. Soc. Exp. Biol. Med. **135**, 68–71 (1970).
24. GELFANT S.: Cancer Res. **37**, 3845–3862 (1977).
25. GIMBORNE M. A., LEAPMAN S. B., COTRAN R. S. and FOLKMAN J.: J. Exp. Med. **136**, 261–276 (1972).
26. GOLDSTEIN L. T., KLIMAN N. R. and MANSON L. A: J. Natl. Cancer Inst. **51**, 1713–1715 (1973).
27. GREEN G. H.: Amer. J. Obstet. Gynaec. **94**, 1009–1022 (1966).
28. HADFIELD G.: Brit. Med. J. **2**, 607–609 (1954).
29. HERSH E. M. *et al.*: Investigation and Stimulation of Immunity in Cancer Patients (G. MATHÉ and R. WEINER, ed.) Springer-Verlag, Berlin, Recent Results in Cancer Research **47**, 25–36 (1974).
30. HIBBS J. B., LAMBERT L. H. and REMINGTON .J. S.: Proc. Soc. Exp. Biol. Med. **139**, 1049–1052 (1972).
31. IGEL H. J., HUEBNER R. J. and TURNER H. C.: Science **166**, 1624–1626 (1969).
32. KELLER R.: Immunology **27**, 285–298 (1974).
33. KELLER R.: Cellular Immunol. **17**, 542–551 (1975).
34. KELLER R.: J. Nat. Cancer Inst. **56**, 369–377 (1976).
35. KELLER R.: Cytostatic and cytocidal effects of activated macrophages. Immunobiology of the Macrophage (D. S. NELSON, ed.) Academic Press, New York, pp. 149–164 (1976).
36. KELLER R.: J. Exp. Med. **138**, 625–644 (1973).

37. KIRCHNER H., HERBERMAN R. B., HOLDEN H. T.: J. Natl. Cancer Inst. **55**, 971–975 (1975).
38. KIRCHNER H., MUCHMORE A. V. and CHUSED T. M.: J. Immunol. **114**, 206–210 (1975).
39. KELIN W. J.: J. Immunol. **109**, 51–58 (1972).
40. KLEIN E. and LINDER O.: Transplant. Bull. **27**, 457–460 (1961).
41. LAMON E. W.: Bioch. Biophys. Acta **355**, 149–176 (1974).
42. LESPINATS G. and POUPON M. F.: Cancer Res. **37**, 1727–1732 (1977).
43. MACLENNAN I. C. M.: Transplant. Rev. **13**, 67–73 (1972).
44. MONTGOMERY T. R., WHITLOCK G. F., NOHLGREEN J. E. and LEWIS A. M.: J. Urol. **86**, 655–658 (1961).
45. MORTENSEN J. D., WOOLNER L. B. and BENNETT W. A.: J. Clin. Endocrinol. Metab. **15**, 1270–1280 (1955).
46. MUNSIE W. J. and FOSTER E. A.: Cancer **21**, 692–698 (1968).
47. NOBLE R. L. and HOOVER L.: Cancer Res. **35**, 2935–2941 (1975).
48. OLD, L. J., BOYSE E. A., CLARKE D. A. and CARSWELL E. A.: Ann. N. Y. Acad. Sci. **101**, 80–106 (1962).
49. OLD L. J., STOCKERT E., BOYSE E. A. and KIM J. H.: J. Exp. Med. **127**, 523–539 (1968).
50. REMINGTON J. S., LAMBERT L. H. and KRAHENBUHL J. L.: Cellular Immunol. **25**, 279–293 (1976).
51. RIGGINS R. S. and PILCH Y. H.: Cancer Res. **24**, 1994–1996 (1964).
52. SENDO F., AOKI T., BOYSE E. A. and BUAFO C. K.: J. Natl. Cancer Inst. **55**, 603–609 (1975).
53. SCHABEL F., FINK M. and GLATZI J.: Paediatr. Paedol. **11**, 417–422 (1976).
54. SENIK A., DEGIORGI E. and GOMARD E.: Int. J. Cancer **14**, 396–400 (1974).
55. SJOGREN H. O. and BORUM K.: Cancer Res. **31**, 890–900 (1971).
56. SJOGREN H. O., HELLSTROM I., BANSAL S. C. and HELLSTROM K. E.: Proc. Natl. Acad. Sci. **68**, 1372–1375 (1971).
57. STJERNSWARD J.: J. Natl. Cancer Inst. **40**, 13–22 (1968).
58. SUGARBAKER E. V., KETCHAM A. S. and COHEN A. M.: Cancer **28**, 545–553 (1971).
59. TAYLOR G.: Brit. Med. J. **1**, 455–462 (1959).
60. TOTH B.: Proc. Soc. Exp. Biol. Med. **112**, 873–875 (1963).
61. VAAGE J.: Cancer Res. **31**, 1655–1662 (1971).
62. WEINHOLD K. J., GOLDSTEIN L. T. and WHEELOCK E. F.: Nature **270**, 59–61 (1977).
63. WHEELOCK E. F.: Personal communication.
64. WHEELOCK E. F., CAROLINE N. L. and MOORE R. D.: J. Virol. **4**, 1–6 (1969).
65. Yu A., WATTS H. and JAFFE N.: New Engl. J. Med. **297**, 121–127 (1977).
66. ZEMBALA M., PTAK W. and HANCZAKOWSKA M.: Clin Exp. Immunol. **15**, 461–466 (1973).